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Tetrazine Mediated Post-polymerization Modification

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ABSTRACT. A new and highly efficient polymer, post-polymerization, modification platform based on an inverse electron demand Diels-Alder reaction is reported. Well-defined defined polymers were synthesized from allyl glycidyl ether and glycidol by anionic ring-opening polymerization with post-polymerization modifications conducted with a number of tetrazine derivatives which carried functional groups spanning from carboxylates and esters to primary amines. Analysis of polymerization kinetics by real-time ¹H NMR and GPC revealed a rapid and high degree of side-chain conversion (>99%), with the generation of well-defined functional polymers, in both organic and aqueous solvents, without the need for additives or catalysts.

Introduction

Poly (ethylene glycol)s (PEGs) are one of the most widely used polymers, with applications ranging from drug-delivery¹ and protein bioconjugation,² to the synthesis of hydrogels,³ as well as being applied in emerging technologies such as solar cells.⁴ Poly (allyl glycidyl ethers) (PAGE's) have been suggested as functional alternatives to PEG, as they offer an identical

polyether backbone, while offering post-polymerization modification capability through the functional allyl handles positioned along the polymer chain.⁵⁻⁹

Post-polymerization modifications are typically based on the reaction of functional groups incorporated within a polymer that are inert under polymerization conditions, but which can be converted in a subsequent reaction step into a broad range of other functional groups, or act as the site of a second polymerization reaction. These modifications need to be highly efficient to guarantee near quantitative conversion, ideally under mild reaction conditions that avoid degradation of either the polymer or the functionality that is being introduced. Although a number of successful reactions have been introduced for post-polymerization modifications,^{10,11} the development of efficient synthetic protocols to decorate polymers with functional units remains a key need in macromolecular chemistry.

The advent of so-called “click chemistry”¹² techniques have led to widely accessible, and well-defined macromolecular architectures and functional polymers. Among these, the copper-catalyzed azide-alkyne Huisgen 1,3-dipolar cycloaddition (CuAAC) reaction has become almost synonymous with the term “click reaction” and has led to a great number of post-polymerization modifications over the past decade.¹⁰ Although the requirement for a metal catalyst and its subsequent removal can be a limitation (particularly for biomedical applications) there are always concerns over the explosive nature of low-molecular weight azido species. Some metal-free “click chemistries” have been reported for polymer modification, such as the ‘thiol-ene’^{13,14} reaction, however, thiol-containing compounds undergo various of oxidation-based chemistries, such as disulfide formation, limiting scope. An other possible drawback of thiol-ene addition chemistry is that the radical formed following thiol addition may attack adjacent vinyl groups, leading to intramolecular cyclizations.¹⁵

The additive free, inverse electron demand Diels-Alder ($_{inv}$ DA) reaction, between electron deficient tetrazines and electron rich dienophiles has attracted considerable attention. Thus tetrazine-norbornene chemistry has been extensively employed in bioconjugation,¹⁶⁻¹⁸ in cellular imaging,¹⁹ and DNA ligation roles.²⁰ Notably, the $_{inv}$ DA has been applied to modify and conjugate polymer chains, however, to date, only tetrazine strained-norbornene chemistry has been reported.²¹⁻²⁶ An alternative to strained norbornenes are unstrained dienophiles which can also react with tetrazines,²⁷⁻²⁹ and which are synthetically readily available, stable, and comparatively small in size compared to norbornene, an important feature as smaller functional side chain typically result in increased degrees of functionalization,³⁰ due to diminished steric effects. In particular, the study of the explosive properties of tetrazine-based high nitrogen molecules indicated that 1,2,4,5-tetrazine derivatives display good thermal stability.^{31,32}

The significant promise of PAGE-based materials, thus promoted an investigation into post-polymerization modification through $_{iva}$ DA reaction's with tetrazines with the aim of establishing a robust platform amenable to the elaboration of polymers with a wide variety of functionalities^{24,33} in an efficient fashion.

Results and Discussion

A series of tetrazine derivatives containing pyridyl, phenyl and/or pyrimidyl moieties attached to carboxylic acids, esters and primary amine moieties were employed to study how these substituents influenced the bimolecular reaction of the $_{iva}$ DA reaction, with allyl glycidyl ether used as the reaction partner in these initial scoping reactions with reaction monitoring conducted via NMR analysis. The reaction with TZ1 was found to proceed to completion within 2 hours at 25 °C (Table 1). Under the same conditions the reactions with the TZ2-TZ7 were significantly

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3 slower with conversions of 17% to 50% within 14 hours, however increasing the temperature to
4
5 60 °C gave conversions of over 90% in 10 hours in all cases. At 100 °C, the reaction with TZ7
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7 reached completion within 3 hours, however under these conditions TZ2 gradually degraded.
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10 TZ2, TZ6 and TZ7 were chosen as examples of different tetrazine reaction centers with rate
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12 constants determined under pseudo first-order conditions following the decay in the absorption
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14 of the tetrazine unit at 530 nm. Pseudo first-order rate constants were obtained by fitting the data
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16 with a simple exponential equation. (Figure S5-7 in supporting information) with the second-
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18 order rate constants were calculated from the linear dependence of the observed rate constants on
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20 the concentration of the allyl glycidyl ether used (Table 2). It was expected that the reaction
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22 between the tetrazines (TZ2-7) and allyl glycidyl ether would be a slower than the corresponding
23
24 strained dienophiles, such as norbornenes ($k \approx 1\text{-}5 \text{ M}^{-1}\text{s}^{-1}$) or *trans*-cyclooctenes ($k \approx 10^4 \text{ M}^{-1}\text{s}^{-1}$).
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26 but there is considerable discrepancy in the literature.^{16,34-37} The strong electron-withdrawing
27
28 effect of the esters in TZ1, clearly enhances the reactivity of the tetrazine, displaying rates
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30 comparable to those of the strained dienophiles with dipyridyltetrazine.³⁴ Herein, our results
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32 showed that the reactivity of TZs followed the order of TZ1 >> TZ2 \approx TZ6 > TZ7, as a result of
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34 the decreasing electron-density of the tetrazines.³⁸ However, TZ1 was highly reactive, being
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36 fully/rapidly degraded in the presence of trace amounts of water and was not used in studies
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38 going forward.
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45 Based upon this understanding, post-polymerization modifications of the poly (allyl glycidyl
46
47 ether)s were carried out. The homopolymers (PAGE1, PAGE2 and PAGE3) and the random
48
49 copolymers poly (allyl glycidyl ether-*co*-glycidol) (PAGE-*co*-PG) were prepared via anion ring-
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51 opening polymerization using potassium alkoxide/naphthalenide as initiators.^{6,7,39,40} All polymers
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obtained exhibited mono-modal molecular weight distributions and low PDIs, with absolute molecular weights determined by ^1H NMR analysis (Table 3).

Similar rates were found when react TZ6, TZ2 and TZ7 with the monomer, AGE at 60 °C (see Figure 1a). The reaction of PAGE2 with TZ2, TZ6 and TZ7 showed relative rates analogous to those found above with under the same reaction conditions (Figure 1b), with TZ2 and TZ6 displaying similar reactivities some 2 fold greater than TZ7. The different rates were likely to be the result of the electronic effects and steric effects of the becoming neighboring tetrazine groups on the polymer chain. It has been reported that steric effects are important when functional groups are close to the polymer backbone.⁴¹ TZ6 is smaller than TZ2 and TZ7, and the pyridine groups of TZ6 can be readily protonated which probably enhances its reactivity, thus resulting in a faster rate of reaction. TZ2 has a bulky end group (tert-butyloxycarbonyl) compared to TZ7 which has a free carboxylic acid group. We assume that the slower rate of conversion with TZ7 was due to the electronic effects of TZ7 with unfavorable carboxylic acid concentrations along the polymer backbone distanced.

Virtually complete conversion of all allyl-ether pendants could be achieved by tuning the reaction temperature (see Figure 2), with NMR revealing complete consumption of all pendant allyl groups within 18 hours at 60 °C and 8 hours at 100°C, with the slowest reacting tetrazine TZ7. Interestingly, the rate of the post-polymerization reaction was also found to be molecular weight dependent, as shown in Figure 3, with PAGE1-3 (M_n = 511 Da, 9 kDa and 40.5 kDa) showing 50% conversion after 1, 4 and 16 hours respectively, despite there being a 4 fold excess of tetrazine (over alkene) in each case. GPC showed the expected increase in molecular weight with no observable crosslinking (Figure 4), It is also important to emphasize that gel permeation

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3 chromatography (GPC) analysis of the resulting polymers showed low PDI = 1.03-1.07,
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5 indicating well-controlled reactions (Table 3).
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8 During post-polymerization modification of PAGE2 with TZ2, the reaction lead to polymers
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10 bearing both hydroxyridazine and 1,2-pyridazine pendant units (as evidenced by ^1H NMR
11 analysis (Figure 5)), over time the aromatization of the intermediate product, hydroxyridazine
12 pendant units, proceeded to completion via air oxidation (O_2).
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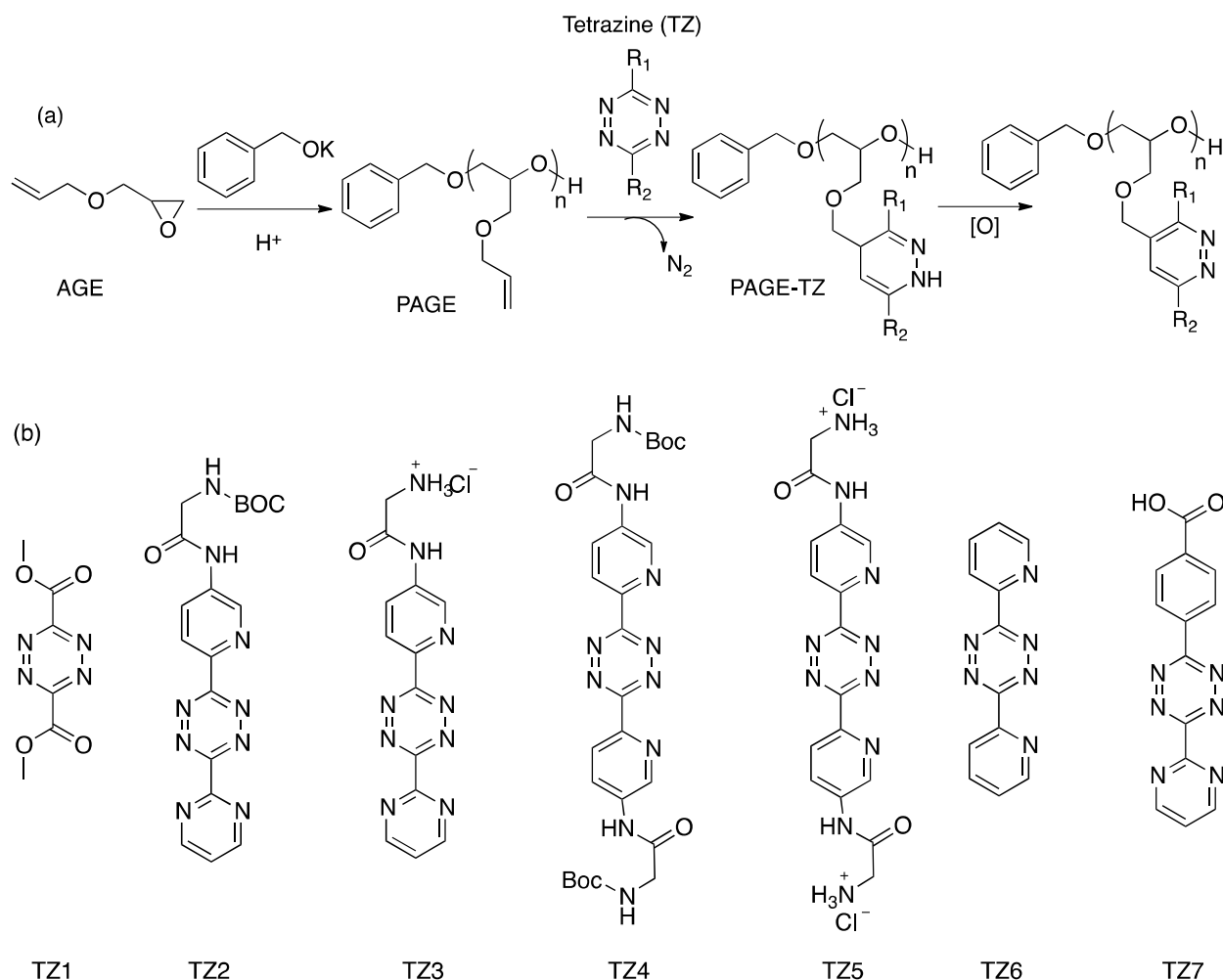
17 To further explore the potential scope of the post-polymerization in aqueous environments, a
18 copolymer was prepared using ethoxyethyl glycidyl ether (EEGE) and allyl glycidyl ether. Poly
19 (allyl glycidyl ether-*co*-glycidol) was obtained through the removal of the protecting group with
20 modification using the water soluble tetrazine, TZ3, conducted in D_2O and followed by ^1H NMR
21 with a single isomer depicted. The reactions proceeded at higher rates compared to similar
22 reactions in $[\text{D}_6]\text{DMSO}$ with the reaction in water reaching full conversion of the polymer within
23 2 hours at 37 °C (Figure 6). The inverse electron demand Diels-Alder performed an aqueous
24 acceleration behavior, in an agreement with the previous report.⁴²
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38 Conclusion

39 A series of allyl glycidyl ether polymers were prepared by anionic ring-opening
40 polymerization giving control over the properties of the polymers. Post-polymerization
41 functionalization of the polymers though an inverse electron demand Diels-Alder reaction with a
42 range of tetrazines was demonstrated in both organic and aqueous environments, giving high
43 degrees of conversion (> 99%) with relative reaction rates determined by ^1H NMR. The
44 observation of different reaction rates between polymers with the tetrazines being a combination
45 of steric and electronic effects, with presumably the steric effects being more following
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modification of the side-chains of the polymer. This approach enables the direct post-functionalization of allyl glycidyl ether polymers in a highly efficient fashion without the need for catalysts or additives. In particular, with high rates of reaction in water, we propose that the reaction offers some advantages over existing methodologies for post-polymerization in aqueous solution. We believe this concept can be used as a versatile post-polymerization modification tool and adds value to the available repertoire of functionalization techniques. The general nature of the synthesis is remarkable and points towards broad applicability of this method in the fabrication of functional soft materials.



Scheme 1. (a) Preparation of poly(allyl glycidyl ether) and their reaction with tetrazine, and (b) Tetrazines used in this study.

Table 1. Reaction conditions used for the analysis of tetrazines with allyl glycidyl ether (AGE) in [D₆]DMSO^a, and conversion.

Tetrazine	Temperature / °C	Conversion ^b / %	Time / h
TZ1	25	100	1.5
TZ2	25	50	10
TZ2	60	91	10
TZ2	100	80	2.5
TZ3	25	38	14
TZ3	60	95	12
TZ4	25	17	10
TZ4	60	95	11
TZ6	25	14	14
TZ6	60	97	14
TZ7	25	32	14
TZ7	60	88	12
TZ7	100	100	3

[^a]the molar ratio of tetrazine to allyl glycidyl ether was 4:1; [^b]Measured by relative integration of the allyl vs aromatic protons of 1,2,4,5-tetramethylbenzene (used as an internal standard).

Table 2. Second-order rate constants for invDA reaction of tetrazines TZ2, TZ6 and TZ7 with allyl glycidyl ethers.

Tetrazine	$k \times 10^{-5} [\text{M}^{-1} \text{s}^{-1}]^{\text{a}}$	R^2
TZ2	8.93	0.97
TZ6	7.18	0.98
TZ7	1.99	0.98

[a]All reactions were performed in DMSO at 25 °C and repeated at least three times. The initial concentration of tetrazines was 0.5 mM and varying concentration of AGE between 5 mM to 50 mM, corresponding to 10-100 equivalents with respect to the tetrazines respectively. The loss of the absorption of the TZ was measured over time at 530 nm. The linear dependence of the observed rate constants k' on the concentration of AGE provided the second-order rate constants k ($n=3$) (for details see the supporting information).

Table 3. Polymer characterization

Polymer	M_n (g mol^{-1})	PDI^{b}
PAGE1	511 ^a	1.06
PAGE2	9004 ^a	1.11
PAGE3	40584 ^a	1.24
PAGE- <i>co</i> -PG	9966 ^a	1.30
PAGE2 + TZ2	10400 ^b	1.07
PAGE2 + TZ6	6430 ^b	1.05
PAGE- <i>co</i> -PG + TZ3	11023 ^b	1.31

[^a]Determined by ^1H NMR. [^b]Determined by GPC (eluting with DMF with 1% LiBr at 60 °C, calibrated with PMMA standards).

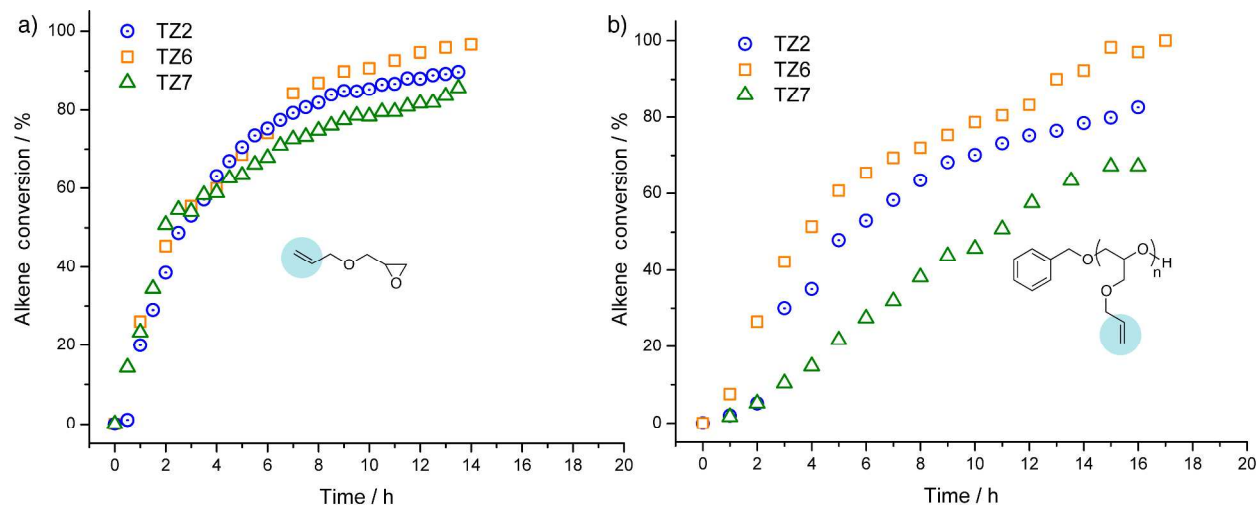


Figure 1. Reaction progress of tetrazines (● TZ2; □ TZ6; and △ TZ7 with a) AGE and b) PAGE2, in $[D_6]$ DMSO at 60 °C. Reaction condition: $[Alkene]/[TZ2] = 1/4$, $[alkene] = 14$ mM.

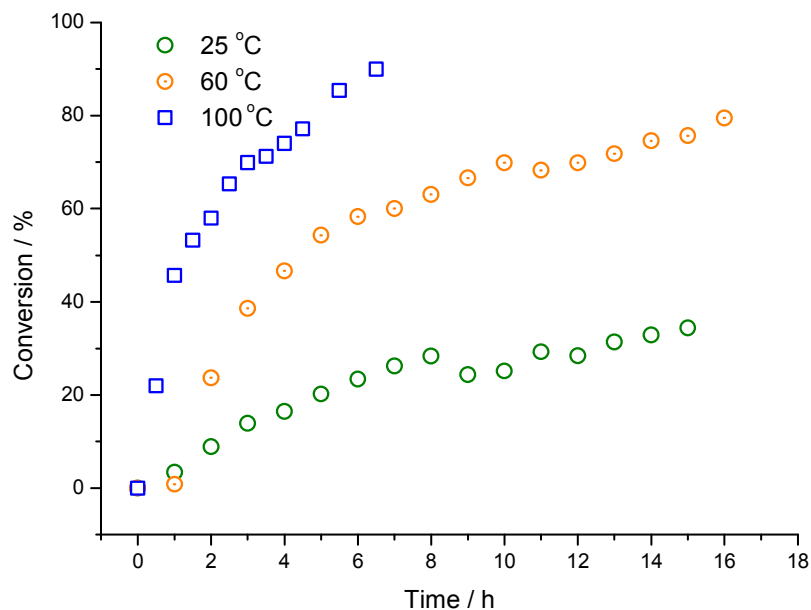


Figure 2. Reaction progress of TZ7 with PAGE2, $M_n = 9004$ g mol⁻¹) in $[D_6]$ DMSO at different temperatures: (●) 25 °C; (○) 60 °C; (□) 100 °C. Reaction condition: $[Alkene]/[TZ2] = 1/4$, $[alkene] = 14$ mM.

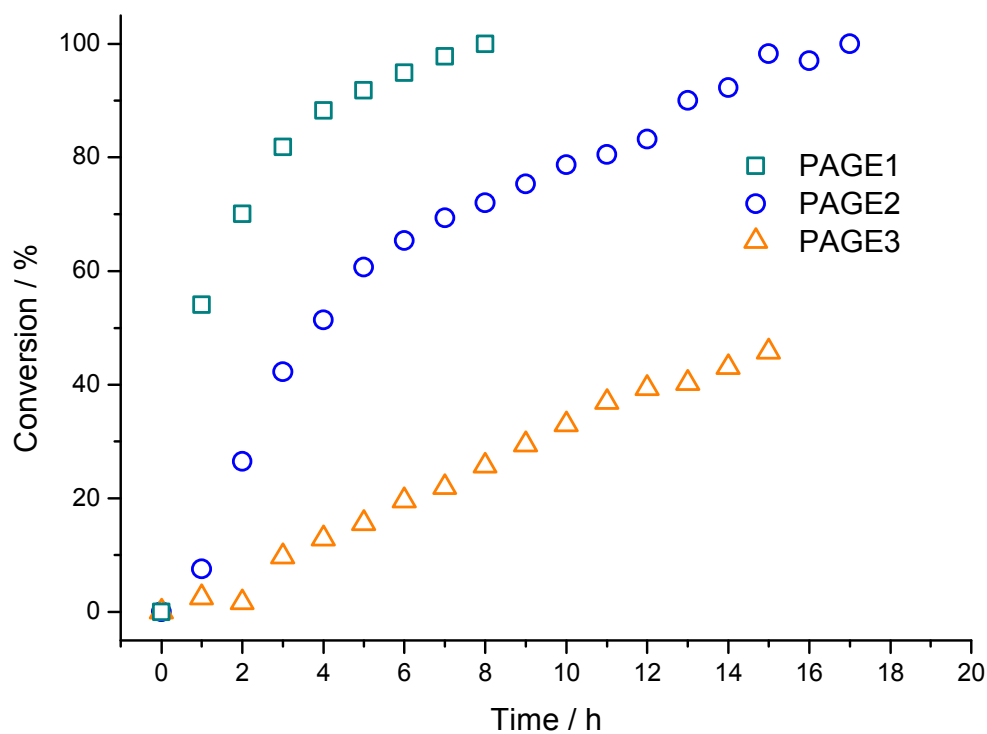


Figure 3. Reaction progress using polymers of differing molecular weigh: (\square) PAGE1; (\circ) PAGE2; and (\triangle) PAGE3 with TZ2 in $[D_6]$ DMSO at 60 °C. $[Alkene]/[TZ2] = 1/4$, $[alkene] = 14$ mM.

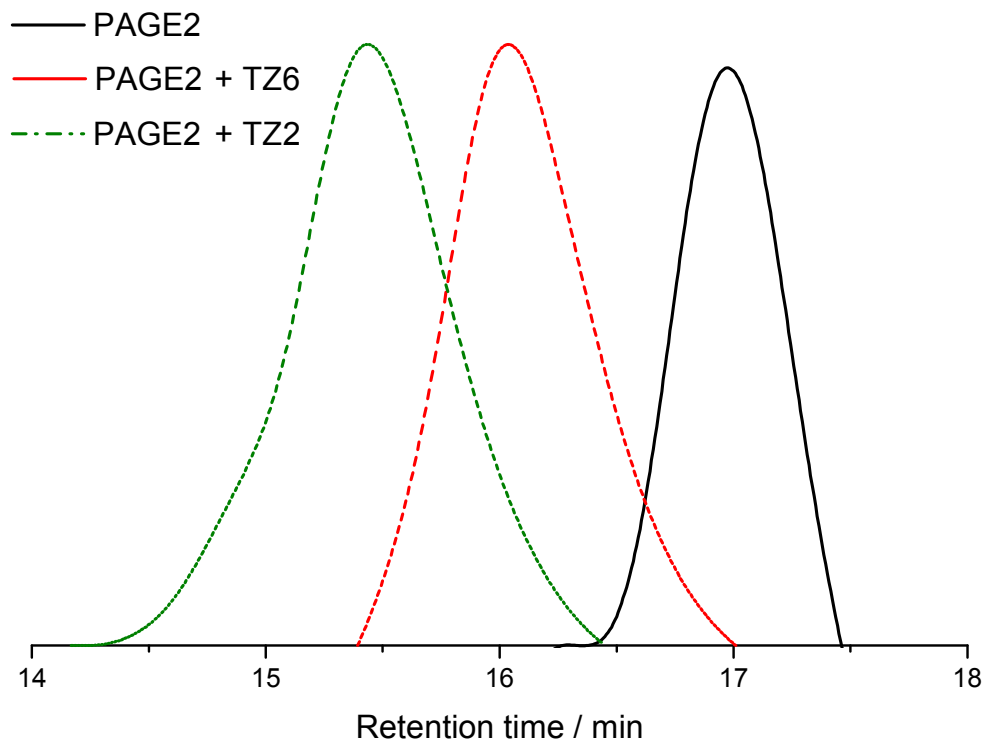


Figure 4. Gel permeation chromatographs of PAGE2 (black trace) and *inv*DA reaction products PAGE2+TZ2 (green trace) and PAGE2+TZ6 (red trace) (eluting with DMF with 1% LiBr at 60 °C, calibrated with PMMA standards).

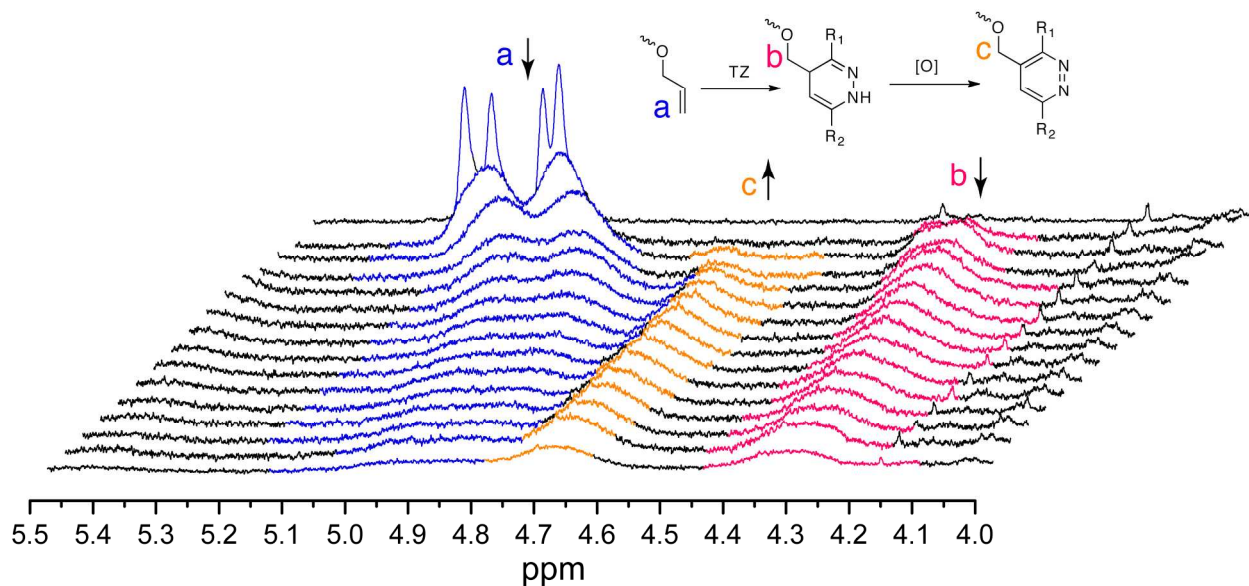


Figure 5. ¹H NMR analysis of the reaction of PAGE2 with TZ2 at 60 °C in [D₆]DMSO. Protons of the alkenes are labeled (a, blue). The hydroxyl methyl protons of the hydroxyridazine, and pyridazine are depicted (b, pink) and (c, brown), respectively. Spectra were recorded at 30 min intervals.

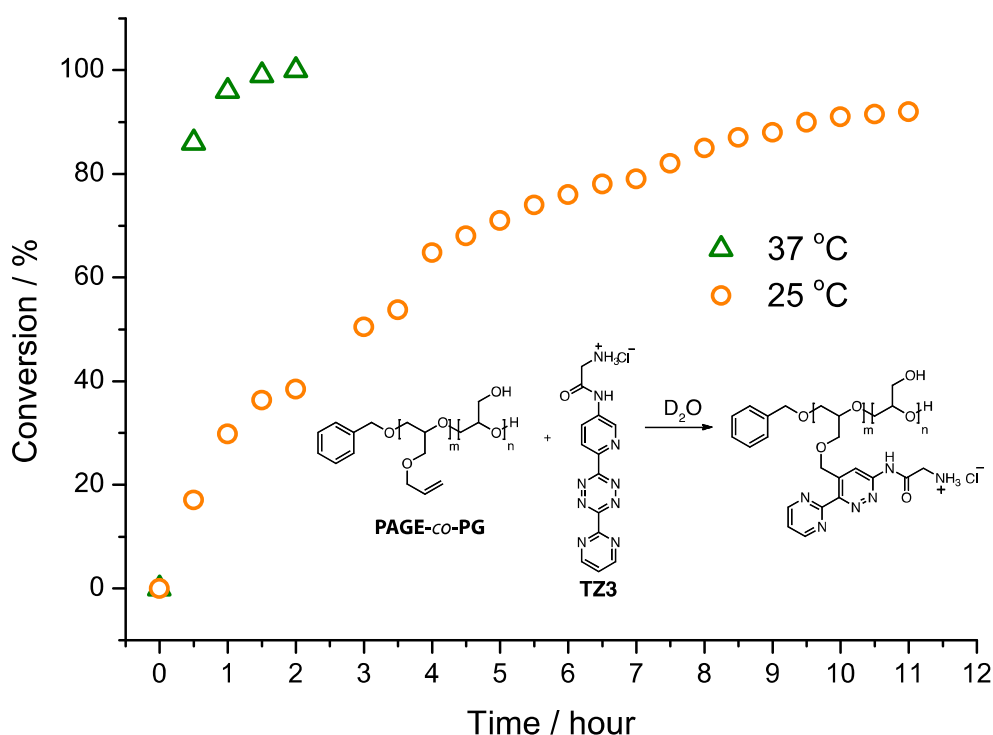


Figure 6. Reaction progress of PAGE-co-PG with TZ3 in D₂O at 25 °C (●) and 37 °C (Δ).

Reaction condition: [Alkene]/[TZ2] = 1/4, [alkene] = 14 mM.

ASSOCIATED CONTENT

Supporting Information. Data analysis and details of synthesis of tetrazines, homopolymers and copolymer. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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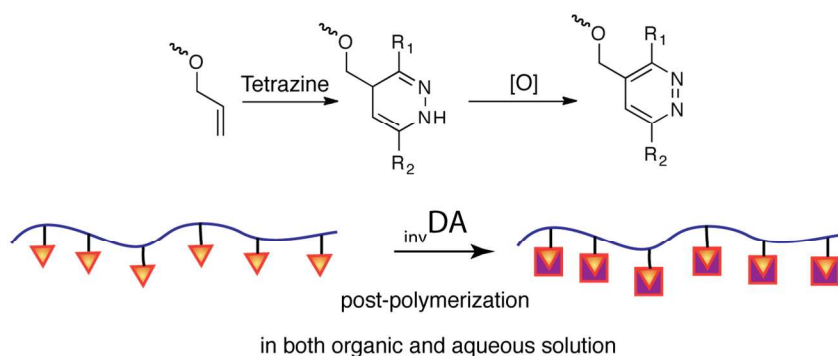
Author Contributions

‡These authors contributed equally.

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